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1. The purpose of this report is to present data on the evaluation of drugs and vaccines in the human malaria/*Aotus lemurinus lemurinus* monkey model experimentally infected *with Plasmodium falciparum* or vivax. During the course of these experiments multiple drug resistance of the highly pathogenic C2A clone of *P. falciparum* was observed when Aotus were treated with Mefloquine (WR142490) at 40 mg/kg orally once. Quinine (WR297608) at 10 mg/kg for five days alone or once at 10 mg/kg followed by two days with PS26 (WR283178) at 10 mg/kg; and PS26 alone at 40 mg/kg for three days. The Uganda Palo Alto (UPA) strain of *P. falciparum* could not be adapted to Aotus. Reduction of efficacy and immunogenicity of plasmid DNA vaccines in a prime-recombinant protein boost schedule cannot be attributed to plasmid concentrations in immunized Aotus. PS26 (WR283178, BQ30798) at 2.5-20 mg/kg orally for three days cured *P. falciparum* Indochina I infections in Aotus monkeys. R-66-1 (WR288911, BQ34018) at 1.25-5 mg/kg orally for three days cured infections of *P. falciparum* Indochina I in Aotus. In contrast, GSQ7302 when given orally at 30 mg/kg twice a day for five days did not.

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The purpose of this report is to present data on the evaluation of drugs and vaccines in the human malaria/Aotus lemurinus lemurinus monkey model experimentally infected with Plasmodium falciparum or vivax. During the course of these experiments multiple drug resistance of the highly pathogenic C2A clone of P. falciparum was observed when Aotus were treated with Mefloquine (WR142490) at 40 mg/kg orally once. Quinine (WR297608) at 10 mg/kg for five days alone or once at 10 mg/kg followed by two days with PS26 (WR283178) at 10 mg/kg; and PS26 alone at 40 mg/kg for three days. The Uganda Palo Alto (UPA) strain of P. falciparum could not be adapted to Aotus. Reduction of efficacy and immunogenicity of plasmid DNA vaccines in a prime-recombinant protein boost schedule cannot be attributed to plasmid concentrations in immunized Aotus. PS26 (WR283178, BQ30798) at 2.5-20 mg/kg orally for three days cured P. falciparum Indochina I infections in Aotus

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INTRODUCTION:

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa **(4)**. During the past 10-20 years the malaria problem has intensified in some parts of the world because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries **(7)**.

The use of *Aotus lemurinus lemurinus* (Panamanian *Aotus* monkey), kariotypes VIII and IX **(16)** as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976, due in part to the availability of this monkey in Panama **(20)**, and also to the increasing drug resistance exhibited by the highly pathogenic *Plasmodium falciparum* parasites in Asia, Africa, and Latin America, and more recently *Plasmodium vivax* in the Melanesian and Indonesian archipielago **(21)**. Previously, Schmidt **(26,27)** used the Colombian *Aotus* as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of *Aotus* for biomedical research in the United States, and in 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian *Aotus* were available for research. Seven strains of *P. falciparum*, Vietnam Smith, Uganda Palo Alto, Vietnam Oak Knoll (FVO), Indochina I, Camp, Santa Lucia **(5)**, and a C2A mefloquine resistant clone, and three strains of *P. vivax* Chesson (chloroquine sensitive), New Guinea AMRU-1 (chloroquine resistant) and Sal-1, have been adapted to Panamanian Aotus. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents. The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia **(25)**. Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however; significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* **(2, 18)**. Desferrioxamine, an iron-specific- chelating agent, was shown to suppress parasitemias of the virulent

Uganda Palo Alto strain of *P. falciparum* **(23)**. The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys **(22)**.

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum, in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine **(17)**. Other *in vitro* studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria **(14)**. Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin, a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo **(1)**. Parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(15)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* **(28)**.

Some strains of *P. vivax* from Melanesia and the Indonesian archipelago have demonstrated resistance to treatment with chloroquine **(19, 24)**. Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys, it was demonstrated that WR238605 is an alternative treatment for chloroquine-resistant vivax malaria **(21)**. The compound WR 238605 is a primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine. Recent studies done at Gorgas Institute with Artemisin derivative drugs developed by the U.S. Army such as Artelinic acid demonstrated its efficacy against the FVO strain of *P. falciparum* when administered orally to *Aotus l. lemurinus.*

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz. to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective plasmid DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of *P. falciparum*. If successful, it will establish, for the first time, that plasmid DNA vaccines can protect non-human primates, a critical step forward for the use of plasmid DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one or more points: a) anti-sporozoite antibodies that prevent invasion of hepatocytes. b) cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes. c) antimerozoite antibodies that prevent invasion of erythrocytes. d) antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses. e) antibodies that attack infected erythrocytes. f) cytokines that kill parasites within erythrocytes and g) anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccines have shown that the Panamanian *Aotus*\|*P. falciparum* model to be suitable for this purpose. **(8-10)**.

Immunogenicity studies of a plasmid DNA vaccines encoding the circumsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys, demonstrated that the intradermal route of inoculation (ID) induces a higher level of antibodies than the intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4)**. We have used this immunization scheduled to test single or multi-gene DNA plasmid vaccines in *Aotus* monkeys. Other experiments, tested the ability of recombinant cytokines GM-CSF to enhance the immunogenicity and protective efficacy of the DNA vaccines.

During the course of these experiments, we have also demonstrated that synthetic oligodeoxynucleotides containing CpG motifs enhance immunogenicity of a peptide malaria vaccine when tested in Panamanian Aotus **(11)**. Also, different vaccine formulations, routes and methods of administration with a comparable Hepatitis B Plasmid DNA vaccine were explored in Panamanian Aotus in order to elucidate the best route and methods of immunization for a plasmid DNA malaria vaccine **(6)**. Further studies with multiple plasmids encoding EBA-175, MSP-1 and AMA-1 did not

induced antigenic competition when this vaccines were delivered as a mixture in Panamanian Aotus **(31)**.

Recently, we tested the hypothesis that *a P. falciparum* ligand, EBA-175 region II (RII), can be used as an immunogen in Aotus to induce antibodies that block the binding of RII to erythrocytes and thus inhibit parasite invasion of erythrocytes **(29)**. When this parasite protein was tested in Panamanian *Aotus l. lemurinus* using a plasmid DNA prime-recombinant protein boost approach, no protection was obtained against a *P. falciparum* FVO strain challenge. However. in the same experiment those animals immunized with a recombinant $MSP1_{42}$ protein/peptide vaccine formulation were partially protected (2001 annual report). These results contrast with those obtained previously in *Aotus nacymae* from Peru were partial protection was achieved using the same EBA-175 region II (RII) formulation and approach **(30)**.

Sterile immunity was achieved by repeated blood stage infections with *P. falciparum* in Aotus monkeys previously immunized with early generation plasmid DNA vaccines AMA-1 or MSP-1 and non-immunized, but no association was found between immunization status and mean pre-patent period. These animals were protected against and homologous and partially protection against an heterologous challenge **(12)**. Also, evaluated in Aotus monkeys were the characteristics of *P. falciparum*-induced anemia in two different experimental settings were a non-antibody/non-complement-mediated lysis of uninfected erythrocytes seems to be the principal cause of anemia and bone marrow suppression and lysis of infected erythrocytes contributed to the anemia **(13)**.

The purpose of this report is to: 1) Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus l. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*, and 2) data on plasmid DNA and recombinant protein malaria vaccine experiments. The U.S. Army and the U.S. Navy Malaria Programs supported these studies.

BODY:

I. Experimental Methods

The first aim of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of *Aotus* experimentally infected with *P. falciparum* (or *P. vivax*). Specifically, the vertebrate host is *A. l. lemurinus*, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in *Aotus*. The Vietnam Smith/RE strain of *P. falciparum* was adapted to *Aotus* of Colombian origin in 1971 **(26)** and in Panamanian *Aotus* in 1976. **(25)**. The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian *Aotus* **(25)**. This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine **(27)**.

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated *Aotus* was diluted appropriately in RPMI, such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. **(3)**

Blood films from untreated *Aotus*, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescence occurred during a 100-day examination period, the infection was considered cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water-soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8° C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression without clearance. The day of clearance was defined as the first of three consecutive days in which the thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

The second objective of this project is to evaluate plasmid DNA vaccines against the blood and sporozoite stages of *P. falciparum* and against the blood stages of *P. vivax* in the Panamanian *Aotus* model. To this end we have evaluated single and multigene DNA vaccines of *both P. falciparum* and *P. vivax* with or without the addition of cytokines. The results of these experiments are detailed in results.

II Results

1. Adaptation of mefloquine resistant *Plasmodium falciparum* C2A clone to Aotus monkeys.

Mefloquine resistant strains of *P. falciparum* have been detected along the Cambodian-Thailand border. These strains have been studied *in vitro* and we have adapted it to splenectomized Aotus as reported previously. The purpose of this experiment was to further passage the splenectomized Aotus *P. falciparum* C2A adapted strain into intact Aotus and treat their infections with several experimental drugs as a preliminary step towards future drug experiments. On May 2, 2002 a splenectomized Aotus (MN12801) was inoculated with a frozen stock of the C2A strain intraperitoneally (IP). This strain which had been passage four times previously in splenectomized Aotus; demonstrated multiple drug resistance to Mefloquine (WR142490) at 20 and 40 mg/kg orally once, Artelinic Acid (WR255663) at 8 and 16 mg/kg and Quinine (WR297608) at 20 mg/kg x 2 days. This animal became patent on day 7 post-inoculation (PI) and its parasitemia peak on day 25 PI at 113,520 parasites x *ul*. Treatment was initiated on day 26 PI with Mefloquine (WR142490) at 20 mg/kg orally once. Parasitemia was suppressed and again treated on day 33 PI this time with Quinine (WR297608) at 20 mg/kg x 5 days without effect. Retreatment with Quinine was initiated on day 46 PI x 2 days and continued on day 48 PI with PS26 (WR283178) a new antifolate drug at 40 mg/kg x 3 days orally. Suppression of parasitemia was observed until day 56 PI when the animal died of multiple organ hemorrhages due to malaria. Blood from this animal was passage further into another splenectomized Aotus (MN12907) on May 24, 2002. This time parasitemia peak on day 11 PI at 121,510 parasites x

ul, treatment was initialed with Quinine (WR297608) at 20 mg/kg x 5 days orally on day 14 PI and parasitemia was suppressed. On day 26 PI parasitemia was then treated with PS26 (WR283178) at 20 mg/kg x 3 days orally without effect. This animal developed soft feces on day 36 PI and was treated with Mefloquine (WR142490) at 40 mg/kg orally once on day 38 PI clearing it parasitemia the following day and remaining negative up to 104 days PI. Citrated whole blood from this animal was further passage into another splenectomized Aotus (MN 12804) on June 7, 2002. Peak parasitemia of 72,420 parasites x *ul* was observed on day 6 PI. Treatment with Quinine (WR297608) at 20 mg/kg once and continued with PS26 (WR283178) at 10 mg/kg x 3 days was initiated on day 11 PI. A suppression of parasitemia was observed on the following days. Diarrea was present on day 20 PI and the animal died of multiple organ hemorrhages due to malaria on day 22 PI. Blood from this animal was further passage on June 18, 2002 into another splenectomized Aotus (MN 13112). Peak parasitemia was observed on day 6 PI at 87,750 parasites x *ul* and treatment was started on day 7 PI with PS26 (WR283178) at 40 mg/kg x 3 days. A suppression of parasitemia was observed on the following days. But on day 13 PI the animal had to be treatment with Mefloquine (WR142490) at 40 mg/kg once due to its poor condition. This animal died of multiple organ hemorrhages due to malaria on day 15 PI. No diarrhea was observed in this animal. Whole citrated blood was passage further into an intact Aotus (MN12977) on June 25, 2002 but the animal remained negative for more than 85 days PI. In summary the C2A clone of *P. falciparum* was passage further to its VIII passage level in splenectomized Aotus, demonstrating to be highly pathogenic for Aotus, but non-adaptable to spleen intact animals. Multiple drug resistance was demostrated during this experiment when Aotus were treated with Mefloquine (WR142490) at 40 mg/kg once, Quinine (WR297608) at 10 mg/kg for five days alone or once at 10 mg/kg followed by two days with PS26 (WR283178) at 10 mg/kg; or PS26 alone at 40 mg/kg for three days..

2. Passage of the Uganda Palo Alto (UPA) strain of *Plasmodium falciparum*.

In order to test a new antifolate drug known as PS26 (WR283178) against known pyrimethamine malaria resistant strains, a frozen stock of the UPA strain of *P. falciparum* brought from WRAIR was inoculated intraperitoneally into an Aotus monkey (MN12973) on 28 June, 2002. The animal remained negative for more than 100 days PI.

3. Immunogenicity Optimization of DNA Vaccines in *Aotus*

On 27 May 2002, six Aotus monkeys were immunized with nD-RII = region II of EBA-175 (native codon usage) in a VR1020 plasmid backbone in

order to demonstrate that we can induce satisfactory levels of antibody using DNA malaria vaccines that have never been concentrated beyond 1 to 1.5 mg/ml. In our two most recent DNA prime/Protein boost malaria vaccine trials, the priming immunizations failed to induce levels of antibody equivalent to those in earlier trials. This diminished response to the DNA priming led to a sub-optimal response to the subsequent protein boost resulting in an overall reduction in response to the complete immunization sequence. The reason for this reduction of immunogenicity in the DNA priming immunizations is not known with certainty, but a close review of the protocol and other records focused attention on the concentration of the DNA. These two most recent trials contained groups that received more than one DNA vaccine. To enable us to mix the DNA preparations without creating excessively large volumes, the DNA was concentrated from its usual concentration of 1 to 1.5 mg/ml to 5 to 6 mg/ml. For intradermal injection, monkeys (6 per group) received 500 μg of plasmid DNA in up to 1 ml of phosphate-buffered saline by needle injection (29 gauge insulin syringe). The maximal volume administered in any one intradermal site was 150 μl. Antibody titers were tested in ELISA against Pichia-produced EBA-175 Region II protein. This technique has been previously described. The antibody titer results were evaluated as follows: archived serum samples from the both successful and sub-optimal immunization regimens were tested simultaneously in ELISA with the serum samples obtained in this study. DNA: Plasmid were delivered in PBS at 500 µg/dose, injected id on the lower back. Total volume per dose not to exceed 1.0 ml. The priming immunizations consisted of three immunizations delivered 28 days apart. Boost: None. Challenge: None. The experiment was terminated on August 20. Serological results as shown on table 1 indicated that there were no significant differences when these titers were compared against titers from previous studies. In conclusion the reduction of efficacy and immunogenicity of plasmid DNA vaccines in a prime-recombinant protein boost schedule cannot be attributed to plasmid concentrations in immunized Aotus.

4. Efficacy of PS26 (WR283178,BQ30798) in *Plasmodium falciparum* Indochina I inoculated Aotus.

On August 12, 2002 nine *Aotus l. lemurinus* monkeys were inoculated with 5 x 10^6 parasites of the Indochina I strain of *P. falciparum* in order to test the efficacy of PS26, a new orally active inhibitor of dihydrofolic acid reductase (DHFR) which has significant activity against drug-resistant *P. falciparum* in vitro. PS26 belongs to a new antifolate class of drugs that have been named oxyguanils. Its predecessors PS15 and WR99210 showed in human clinical trials severe gastrointestinal symptoms when given at 200 mg/kg five times a day for three days. The experiment consisted of four groups of two Aotus each and one control When parasitemia reached 5,000

parasites x *ul* the groups received PS26 (WR283178, BQ30798) at 2.5, 5, 10 and 20 mg/kg orally once a day for three days. Daily blood smears were examined for parasitemia. Infections were considered cured if parasitemia remained negative for 100 consecutive days. As shown in tables 2 and 3, parasitemia was cleared on the second day Post Treatment (PT). In groups 1, 2 & 4 which received PS26 at 2.5, 5 and 20 mg/kg orally for three days respectively,. Only in Group 3, that received 10 mg/kg, parasitemia cleared in one animal on the second day PT and on the third day in the other one. The control monkey received mefloquine treatment at 20 mg/kg when its parasitemia reached more than 600,000 parasites x *ul* on the fourth day PT. On day 61 PI one animal from group 3, which received PS26 at 10 mg/kg died of complications due to a large intestinal obstruction. The rest of the animals remained negative and no gastrointestinal complications were observed for more than 100 days PI. In conclusion PS26 (WR283178, BQ30798) at 2.5-20 mg/kg orally for three days cured *P. falciparum* infections in Aotus monkeys.

5. Efficacy of RS-66-1 (WR288911, BQ34018) dihydrofolate reductase (DHFR) inhibitor against infections of *P. falciparum* Indochina I in Aotus Monkeys.

New observations on lack of complete cross-resistance of proguanil analogs and their triazine metabolites has stimulated renewed interest in dihydrofolate reductase (DHFR) inhibitors in combination with dihyropterorate synthase DHPS inhibitors as second and third generation "Fansidar-like" drugs. The recent emergence of multiple DHFR/DHPS mutants in East Africa suggests that LapDap, a second-generation combination of Lapudrine (chlorproguanil) and Dapsone (LapDaP) may have limited utility by the time is can be fielded. These experiments will evaluate and select newer, more potent biguanide candidate drugs in combination with Dapsone for treatment and prophylaxis of multidrug-resistant strains of malaria. On January 23, 2002 ,seven groups of two Aotus each including controls were inoculated with 5 x 10^6 parasites of *P. falciparum* Indochina I strain. The animals were weight weekly and CBC and Chemistry panel (ALT, Creatinine and BUN) analyze once a week after inoculation. When parasitemia reached 5,000 parasites x *ul*. Groups 1-4 received RS-66-1 (WR288911, BQ34018) at 5, 2, 1.25 and 0.65 mg/kg orally once a day for three days. Groups 5 received Dapsone (WR000448, BN: BQ29320) at 40 mg/kg and 2.5 m/kg x two days. Group 6 received Pyrimethamine (BM 16373) at 40 mg/kg once and 2.5 m/kg x two days. Group 7 served as controls. Daily blood smears were examined for parasitemia determination by the Earle and Perez method. Infections were considered cured if parasitemia remains negative for 100 consecutive days. As shown on tables 4-5, all groups cleared their parasitemias on the third day post-treatment, except for one monkey from

group 6 that received pyrimethamine that cleared its parasitemia on the second day post-treatment. Recrudescence occurred on day 20 post-treatment (PT) first in group four in two monkeys which received RS-66-1 at 0.65 mg/kg and on Group 5 and 6 in one monkey each on day 21 PT. At the moment of this report monkeys from Groups 1-4 have remained negative for more than 29 days PT. No significant changes were observed in CBCs or blood chemistries. In conclusion R-66-1 (WR288911, BQ34018) at 1.25-5 mg/kg orally for three days cured infections of *P. falciparum* Indochina I in Aotus.

6. Pharmacokinetics, efficacy and tolerability of GSQ-7302 in Aotus Monkeys.

GeneSoft, Inc. has developed a group of compounds that have a unique method of inhibiting or destroying microscopic human pathogens. These compounds bind to AT-rich areas of DNA, preventing successful growth of the organisms. The general term for these molecules is antigenomic therapeutics. *In vitro* studies have shown several of these antigenomic therapeutic compounds to have anti-malarial activity at extremely low concentrations. Toxicity Screening in a Multiple Dose, 4-Day Mouse Model: Evaluation of GSQ7302 demonstrated that with once daily dosing for each of 4 days, there was no apparent toxicity with GSQ 7302 given IV at 30, 50 or 75 mg/kg. PO administration of GSQ 7302 at 100 mg/kg resulted in a liver:body weight ratio that was 0.84-fold of the ratio observed in the vehicle control treatment. Presumably, PO dosing of GSQ 7302 was associated with enhanced trafficking of GSQ 7302 to the liver, and this accounted for the hepatic effects being specific to the oral route of administration. The objectives of this study were: a). To study the single dose and multiple dose whole blood pharmacokinetics of GSQ-7302 administered orally at 30 mg/kg BID for a total of 5 daysb). To study the tolerability of GSQ-7302 in infected Aotus monkeys and get some primary and secondary efficacy endpoints to identify an appropriate dose/regimen for the definitive efficacy study. On January 23, 2003, two male *Aotus l. lemurinus* MN 13138 and MN 13140 with 920 and 910 grams of weight respectively, were infected with 5×10^6 *P. falciparum* Indochina I parasites. Each monkey received GSQ-7302 daily at 30 mg/kg BID (8 hr apart) for a total of 5 days. Treatment started when parasitemia reached 5,000 parasites x ul. Blood samples for CBC and Chemistry (ALT, Creatinine and BUN) were collected on day 0 and 6 PI. Blood samples for pharmacokinetics were collected on the following time points: Day 0: CBC and Chemistry panel (ALT, Creatinine and BUN). Day 1: Predose, am dose, 20 min, 1 hr, 4 hr, and 7:30 hr, pm dose. Day 4: Predose (am dose); Predose (pm dose). Day 5: Predose, (am dose), 20 min, 1 hr, 4 hr, 7:30 hr, and 24 hr. Day 6: CBC and Chemistry Panel. Animal feeding and weight were documented. CBC and Chemistry panels were done the day prior to first dose and 24 hours after last dose. Mefloquine was

administered at 20 mg/kg when parasitemia reached more than 400,000 parasites x *ul* or Hto was reduced 50% from baseline. As shown on Table 6-7 no effect of the drug was obtained except for a transient suppression of parasitemia in one monkey, which have to be cured due to sustained low-grade parasitemia with >50% reduction in Hto. Diarrhea and soft feces were observed on days 4-5 days PT in one animal. In conclusion GSQ7302 a new drug belonging to a class of antigenomic therapeutic drugs failed to cure *P. falciparum* Indochina I infections in Aotus when given orally at 30 mg/kg twice a day for five days.

KEY RESEARCH ACCOMPLISHMENTS:

1. The C2A clone of *P. falciparum* was passage further to its VIII passage level in splenectomized Aotus, demonstrating to be highly pathogenic for Aotus, but non-adaptable to spleen intact animals. Multiple drug resistance was demostrated during this experiment when Aotus were treated with Mefloquine (WR142490) at 40 mg/kg once, Quinine (WR297608) at 10 mg/kg for five days alone or once at 10 mg/kg followed by two days with PS26 (WR283178) at 10 mg/kg; or PS26 alone at 40 mg/kg for three days.

2. The Uganda Palo Alto (UPA) strain of *Plasmodium falciparum* could not be adapted to *Aotus l. lemurinus*.

3. Reduction on efficacy and immunogenicity of plasmid DNA vaccines in a prime-recombinant protein boost schedule cannot be attributed to plasmid concentrations in the inoculum used. No significant differences were found when titers from this study were compared against titers from previous studies done in Lima, Peru.

4. PS26 (WR283178, BQ30798) a new orally active inhibitor of dihydrofolic acid reductase (DHFR) which has significant activity against drug-resistant *P. falciparum in vitro* cured *P. falciparum* Indochina I infections in Aotus monkeys when administered orally at 2.5-20 mg/kg for three days.

5. R-66-1 (WR288911, BQ34018) a new dihydrofolate reductase (DHFR) inhibitors cured infections of *P. falciparum* Indochina I in Aotus at 1.25-5 mg/kg orally for three days. When given at 0.65 mg/kg orally for three days recrudescence occurred.

6. GSQ7302 a new drug belonging to a class of antigenomic therapeutic drugs failed to cure *P. falciparum* Indochina I infections in Aotus when given orally at 30 mg/kg twice a day for five days.

REPORTABLE OUTCOMES:

I. Manuscripts:

1. Jones TR, Gramzinski RA, Aguiar JC, Kim Lee Sim B, Narum DL, Furhmann SR, Kumar S, Obaldia NIII, Hoffman SL. 2002. Absence of antigenic competition in Aotus monkeys immunized with Plasmodium falciparum DNA vaccines delivered as a mixture. Vaccine 20(11-12):1675-80

2. Jones TR, Stroncek DF, Gozalo AS, Obaldia NIII, Andersen EM, Lucas C, Narum DL. Magill AJ, Sim BKL, Hoffman SL. 2002. Anemia in Parasite- and Recombinant Protein-Immunized Aotus Monkeys Infected with *P. falciparum.* Blood. Am J Trop Med Hyg. 66(6):672-679.

II. Presentations:

1. Obaldia NIII et al. Anemia in *Aotus lemurinus lemurinus* monkeys infected with P. vivax. Vivax Malaria Research: 2002 and Beyond. Siam City Hotel, Bangkok, Thailand. February 3-8, 2002.

2. Obaldia NIII et al. Reversal of chloroquine resistance with the co-administration of prochlorperazine and chloroquine against infections of chloroquine resistant (CQR) AMRU-1 strain of *P. vivax* in *Aotus lemurinus lemurinus* monkeys. Vivax Malaria Research: 2002 and Beyond. Siam City Hotel, Bangkok, Thailand. February 3-8, 2002.

CONCLUSIONS:

1. Multiple drug resistance of the highly pathogenic C2A clone of *P. falciparum* was observed when Aotus were treated with Mefloquine (WR142490) at 40 mg/kg orally once. Quinine (WR297608) at 10 mg/kg for five days alone or once at 10 mg/kg followed by two days with PS26 (WR283178) at 10 mg/kg; and PS26 alone at 40 mg/kg for three days.

2. The Uganda Palo Alto (UPA) strain of *Plasmodium falciparum* could not be adapted to *Aotus l. lemurinus*.

3. Reduction of efficacy and immunogenicity of plasmid DNA vaccines in a prime-recombinant protein boost schedule cannot be attributed to plasmid concentrations in immunized Aotus.

4. PS26 (WR283178, BQ30798) at 2.5-20 mg/kg orally for three days cured *P. falciparum* Indochina I infections in Aotus monkeys.

5. R-66-1 (WR288911, BQ34018) at 1.25-5 mg/kg orally for three days cured infections of *P. falciparum* Indochina I in Aotus.

6. GSQ7302 when given orally at 30 mg/kg twice a day for five days did not cured infections of *P. falciparum* Indochina I in Aotus.

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TABLE 1

IMMUNOGENICITY OF HIGH VS LOW CONCENTRATED PLASMID DNA EBA-175 VACCINE IN AOTUS MONKEYS

Trial Sera	GMT titer Post second Immunization	GMT titer Post third immunization
LP-1	3518	13450
NP41	1212	7171
	*P = 0.367	*P = 0.283

*=Test = independent samples t-test on log-transformed data
LP1= Low concentrated DNA plasmid vaccine. Lima, Peru Trial with *Aotus nacymae*
NP41= Highly concentrated DNA plasmid vaccine. Panama Trial with *Aotus l. lemurinus*

TABLE 2

SUMMARY OF ACTIVITY OF PS26 (WR283178;BQ30798)
AGAINST INFECTIONS OF THE INDOCHINA I STRAIN OF *Plasmodium falciparum* in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
13121	2.5			X	2		100
13116	2.5			X	2		100
13120	5			X	2		100
13114	5			X	2		100
13096	10			X	3		100
13104	10			X	2		100
13117	20			X	2		100
13115	20			X	2		100

TABLE 3

DETAILED PARASITEMIA OF PS26 (WR283178;BQ30798)
AGAINST INFECTIONS OF THE INDOCHINA I STRAIN OF P*lasmodium falciparum* in Aotus monkeys.

MONKEY #	RX INITIATED			PARASITEMIA PER ccmm x 10^3								Days
	DAY P.I	DAY PAT.	MGKG	DAY PRE RX	DAY OF RX 1	DAY OF RX 2	DAY OF RX 3	DAY POST RX 1	DAY POST RX 2	DAY POST RX 3	DAY POST RX 4	Neg
13121	5	5	2.5	0.37	10.8	1.89	0.66	0.01	0	0	0	100
13116	5	4	2.5	0.44	21.5	4.88	1.34	0.28	0	0	0	100
13120	5	5	5	0.37	20.2	2.49	1.03	0.01	0	0	0	100
13114	5	4	5	0.26	19.3	2.51	0.29	0.01	0	0	0	100
13096	5	4	10	0.57	30.8	4.49	0.53	0.4	0.01	0	0	100
13104	5	5	10	1.28	35.7	1.85	0.33	0.01	0	0	0	100
13117	5	5	20	0.62	28.5	3.56	1.32	0.35	0	0	0	100
13115	5	5	20	0.48	19.1	1.19	0.86	0.01	0	0	0	100
13105	CONTROL			0.33	25.5	31.6	182.2	383.25	177.75	608.25*	118.53	0

*=Treated with 20 mg/kg Mefloquine

TABLE 4

SUMMARY OF ACTIVITY OF R-66-1 (WR288911,BQ34018)
AGAINST INFECTIONS OF THE INDOCHINA I STRAIN OF *Plasmodium falciparum* in Aotus monkeys.

MONKEY #	Daily Dose x 3 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
96012	5			X	3		29
13124	5			X	3		29
95003	2			X	3		29
13125	2			X	3		29
97016	1.25			X	3		29
13128	1.25			X	3		29
97006	0.65			X	3	20	19
90031	0.65			X	3	20	19
12954	40*			X	3	21	20
13122	40*			X	3		29
13123	40**			X	3		29
93010	40**			X	2	21	20

*=Dapsone (WR288911,BQ29320) 40 mg/kg
**=Pyrimethamine (BM16373) 40 mg/kg

TABLE 5

DETAILED ACTIVITY OF R-66-1 (WR288911,BQ34018)
AGAINST INFECTIONS OF THE INDOCHINA I STRAIN OF *Plasmodium falciparum* in Aotus monkeys.

MONKEY #	RX INITIATED			PARASITEMIA PER ccmm x 10^3								Days
	DAY P.I	DAY PAT.	MGKG	DAY PRE RX	DAY OF RX 1	DAY OF RX 2	DAY OF RX 3	DAY POST RX 1	DAY POST RX 2	DAY POST RX 3	DAY POST RX 4	Neg
96012	5	3	5	0.24	14.1	1.89	1.49	0.01	0.01	0	0	29
13124	5	3	5	1.32	16.7	1.87	1.5	0.01	0.01	0	0	29
95003	5	3	2	1.1	43.1	9.75	2.8	0.26	0.01	0	0	29
13125	5	4	2	0.88	24.1	19.4	4.3	0.48	0.01	0	0	29
97016	5	3	1.25	2.33	29.4	6.75	6	0.66	0.01	0	0	29
13128	5	4	1.25	1.08	45.3	12.4	3.65	0.35	0.01	0	0	29
97006	5	3	0.65	0.69	20.8	12.01	2.28	0.42	0.01	0	0	19
90031	5	3	0.65	0.46	17.8	18.15	5.12	0.48	0.01	0	0	19
12954	5	4	40*	0.97	24.6	9.1	1.14	0.01	0.01	0	0	20
13122	5	4	40*	0.77	19	13.9	4.78	0.55	0.01	0	0	29
13123	5	4	40**	1.25	7.33	6.79	4.9	0.31	0.01	0	0	29
93010	5	3	40**	1.47	11.5	4.65	1.69	0.01	0	0	0	20
13127	CONTROL	4		1.52	45	38.4	656.8***	170.25	7.45	0.01	0.01	0
13126	CONTROL	3		1.45	33.4	27.54	330.75	329	410.25***	203.25	39.75	0

*=Dapsone (WR288911,BQ29320) 40 mg/kg
**=Pyrimethamine (BM16373) 40 mg/kg x 1 day and 2.5 mg/kg x 2 days
***=Treated with 20 mg/kg Mefloquine

TABLE 6

SUMMARY OF ACTIVITY OF GSQ7302 AGAINST INFECTIONS OF THE INDOCHINA I STRAIN OF *Plasmodium falciparum* in Aotus monkeys.

MONKEY #	Daily Dose x 5 days Mg/Kg	Response of parasitemia to Rx			Days from final Rx to parasite clearance	Days from final Rx to recrudescence	Notes No. of days negative
		None	Suppressed	Cleared			
13138	30 BID		X				0
13140	30 BID	X					0

TABLE 7

DETAILED PARASITEMIA OF GSQ7302 AGAINST INFECTIONS OF THE INDOCHINA I STRAIN OF *Plasmodium falciparum* in Aotus monkeys.

MONKEY #	RX INITIATED			PARASITEMIA PER ccmm x 10^3								Days
	DAY P.I	DAY PAT.	MGKG	DAY PRE RX	DAY OF RX 1	2	3	4	5	DAY POST RX 1	2	Neg.
13138	5	4	30 BID	0.9	47.1	22.75	191.25	90.75	218.25	188.75	152.25	0
13140	5	4	30 BID	1.76	19.88	21	256.5	240.75	434.25*	54.75	0.95	0
13127	CONTROL			1.52	45	38.4	656.81*	170.25	7.45	0.01	0.01	0
13126	CONTROL			1.45	33.45	27.54	330.75	328.5	410.25*	203.25	39750	0

*Treated with 20 mg/kg Mefloquine